Ubiquitin and Ubiquitin-like Modifications in Viral Infection and Immunity
SALK-SPONSORED WORKSHOP
August 28-30,2007

Matthew D. Weitzman, PhD,
Salk Institute, La Jolla, CA
Eun-Chung Park, PhD
Virology Branch, DMID, NIAID, NIH, Bethesda, MD

The ubiquitin-proteasome system (UPS) is involved in virtually every biological process, and so it is not surprising that it plays important roles in both viral infection and host immune defenses. Viruses have evolved many ways to manipulate their host cells in order to facilitate their own replication and maximize progeny production. During the course of infection the invading pathogen encounters many obstacles and host defense systems. Viruses have developed ingenious strategies to either inactivate these defenses or exploit cellular systems that could lead to its destruction. Many of these assaults, defenses, and counter-attacks involve aspects of the ubiquitin system. The objective of this workshop was to bring together researchers in the areas of ubiquitin, virology, and immunology, in order to explore the links between infection and ubiquitin pathways. In four different sessions we heard about how ubiquitin and ubiquitin-like modifications are employed by viruses in their quest to take over the cell, and also by the host as it attempts to defend itself from the viral invasion. Experts in diverse areas were invited to share their latest results on topics revolved around Host Defenses, the role of Cellular Structures, Immune Responses, and Infection, Trafficking and Budding.

The workshop started with an introduction to the cellular UPS and the approximately 900 potential proteins that are involved (Wade Harper). There are numerous examples of viruses that inactivate or exploit the cellular protein modification apparatus to aid infection, and there are multiple steps during the virus lifecycle that are impacted. The first session dealt with cellular defenses such as the role of SUMOylation in the early steps of retrovirus infection (Stephen Goff), inactivation of the antiviral APOBEC protein by HIV Vif (Xiao-Fang Yu), and ways that the viruses employ ubiquitination to manipulate the cellular DNA damage response (Matthew Weitzman). Ubiqutin and SUMO play roles in intracellular movement, targeting and nucleation of cellular structures. This was clearly demonstrated in talks about autophagy (Jennifer Lippincott-Schwartz), cytoplasmic structures formed by the antiviral TRIM proteins (Tom Hope), and disruption of PML nuclear bodies (Roger Everett, Patrick Hearing, Arnold Berk). Ubiquitin modification also plays a role in sorting and trafficking (Juan Bonifacino, Scott Emr), and this is important for both immune responses and viral budding (Wes Sundquist). Beautiful structural studies throughout the meeting provided a picture of viral and cellular proteins coming together to regulate processes by ubiquitin miodification. These included cullin complexes (Ning Zheng) and the endosomal sorting complexes required for transport (ESCRT) that are important in HIV budding (James Hurley, Wes Sundquist).

Host defenses often employ aspects of the UPS to degrade or modify cellular proteins. This was highlighted by the role of ubiquitin in immunes responses and antigen

presentation (James Chen, Jonathan Yewdell). An interesting ubiquitin-like modification involves the interferon-stimulated ISG15, which functions as a critical host antiviral molecule (Deborah Lenschow, Dong-Er Zhang). Viruses also manipulate the cellular systems to favor their own persistence or transformation of host cells. Examples were presented from EBV (Maria Masucci) and HPV (Martin Scheffner). One way around the host defenses is to employ viral encoded ubiquitn-specific proteases, as was shown for herpesvirus (Hiodde Ploegh) and SARS coronavirus (Susan Baker). These proteins might also present attractive targets for viral-specific inhibitors.

On the final day a session on Emerging Technologies covered the very latest techniques and approaches that are being developed to interrogate the ubiquitin machinery. This included genomic approaches that use shRNA libraries to knockdown genes involved in the conjugation and removal of ubiquitin and ubiquitin-like proteins to viral and cellular substrates (Wade Harper). Another approach to identify protein complexes involved in ubiquitin pathways is the use of high throughput proteomic analysis. Mass spectrometry, coupled with stable isotope ratiometric quantification, is being used to quantitate ubiquitin substrates and proteasome turnover (Raymond Deshaies). These systems-level approaches will allow us to map the UPS networks and decipher the complex biology associated with ubiquitin modifications.

The workshop successfully achieved its goal of promoting communication between labs working in diverse but overlapping areas connected with ubiquitin, infection and immunity. It brought together experts in these different fields in a friendly atmosphere that facilitated conversations and interactions with students and postdocs at two very active poster sessions. Viruses have historically been important tools to uncover key regulators of fundamental cellular processes. Further study in these connected fields will help to unravel the mechanisms of specificity and substrate selection by ubiquitin modifiers, and how these modifications are employed to regulate location and function within the cell. It is also hoped that research in this area will identify potential new targets for therapeutic intervention.

Recommendations:

1. A PA/RFA for collaborative projects that look at the role of Ub and UBL pathways in virus infection and immunity
2. A resource similar to the AIDS Reagent Program or the NCI Developmental Therapeutics Program
3. This could provide reagents not commonly commercially available, including collections of:
    a. relevant cDNAs
    b. siRNA or shRNA libraries or pools or individual clones
    c. antibodies
4. A resource for providing chemical inhibitors such as resynthesizing known drugs
5. Screening facilities for siRNA, shRNA, or drug libraries